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Induction of HIV-1 Nef-Specific Cytotoxic T Lymphocytes by Nef-Expressing DNA Vaccine

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Recently, some individuals who have remained seronegative despite definite exposure to HIV-1 have been reported. Among these individuals, an unusually high frequency of HIV-1 Nef-specific cytotoxic T lymphocytes was observed. Direct injection of plasmid DNA encoding foreign antigen can elicit both cell-mediated immunity and antibody responses (DNA vaccine). We constructed an HIV-1 Nef-expressing plasmid, and we induced HIV-1 Nef-specific cytotoxic T lymphocytes. This is the first report of inducing HIV-1 Nef-specific cytotoxic T lymphocytes by DNA vaccine. © 1996 Wiley-Liss, Inc.

Key words: HIV-1, Nef, DNA vaccine, cytotoxic T lymphocyte

INTRODUCTION

Recently, some individuals who have remained seronegative despite definite exposure to HIV-1 have been reported. Among these individuals, unusually high frequencies of HIV-1 Nef-specific cytotoxic T lymphocytes (CTL) were observed [1,2]. It is possible that those with high levels of Nef-specific CTL activity can be protected from the HIV-1 infection.

For HIV-1 vaccine development, Nef protein has some advantages. This is a regulatory protein expressed early in HIV-infected cells. Thus, immune responses directed against Nef protein may be more efficient in the elimination of infected cells before any release of new viral particles. Moreover, lower variability in its sequences than in the envelope results in the existence of fewer HIV-1 variants escaping from CTL recognition.

Except for attenuated virus vaccines, induction of CTL is generally difficult. To induce CTL, it is essential that the antigenic peptide binds with major histocompatibility complex (MHC) class 1 molecules in the endosomes. Direct injection of plasmid DNA into muscle cells driven by cytomegalovirus promoter encoding the *nef* sequence subsequently leads to the expression of its encoding protein in muscle tissues. Thus, this DNA vaccine is capable of efficiently inducing Nef-specific CTL [3,4].

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MATERIALS AND METHODS

DNA encoding the HIV-1 IIIB *nef* region was extracted by polymerase chain reaction (PCR). Sense primer and antisense primer had the following sequences, respectively: 5′-CCCAAGCTTTTGCTATAAGATGGGTGGCAAGTG-3′ with the *Hind*III site, and 5′-CGGGATCCAGAGACCCAGTACAGGCAAAAAGC-3′ with the *Bam*HI site. This PCR product was cloned into the expression plasmid pBC12/CMV, as previously described [3].

Two μg of Nef-expressing DNA vaccine were inoculated into the gastrocnemius muscles of 4-week-old BALB/c mice, and 4 weeks after inoculation, the mice were killed and spleen cells were collected. These splenocytes were restimulated with synthesized Nef-specific T-cell epitopes (QVPLRPMTY and GVRYPLTFGWCYKLVP) [5], and then cultured for 5 days. Cytolytic activity of these cells (effector cells) was measured by the standard 6-hr ^{51}Cr-releasing method. For target cells, P388D$_1$, a cell line derived from a DBA/2 mouse, was

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used. This cell line has an MHC identical to that of BALB/c mice ($H2^d$). These cells were pulsed with 2 μg/ml of each peptide for 60 min, then labeled with $[^{51}Cr]Na_2O_4$, and used as target cells. Anti-Nef-antibody titer was examined by enzyme-linked immunosorbent assay (ELISA), but it was not detectable.

RESULTS

CTL activity toward target cells pulsed with synthesized peptide GVRYPLTFGWCYKLVP had nearly 30% greater ^{51}Cr release than nonpeptide-pulsed target cells. Effector cells prepared from control DNA-injected mice showed no CTL activity. CTL responses to target cells pulsed with QVPLRPMTY had nearly 25% greater ^{51}Cr release.

DISCUSSION

Our data clearly demonstrate the induction of HIV-1 Nef-specific CTL. To date, the injection of recombinant vaccinia virus expressing HIV-1 Nef or bacille Calmette-Guerin (BCG) shuttle vector has been capable of inducing HIV-1 Nef-specific CTL [6]. However, pathogenicity of these vectors cannot be completely denied [7]. To our knowledge, this is the first report of successful induction of HIV-1 Nef-specific CTL by Nef-expressing DNA vaccine, whose pathogenicity is almost negligible.

DNA vaccines are efficiently capable of inducing CTL to the foreign antigen. These results are further applicable to chronic and persistent viral infections such as HTLV-1 or hepatitis C. In these viral infections, antibody responses are rather ineffective, and CTL activities are clearly needed.

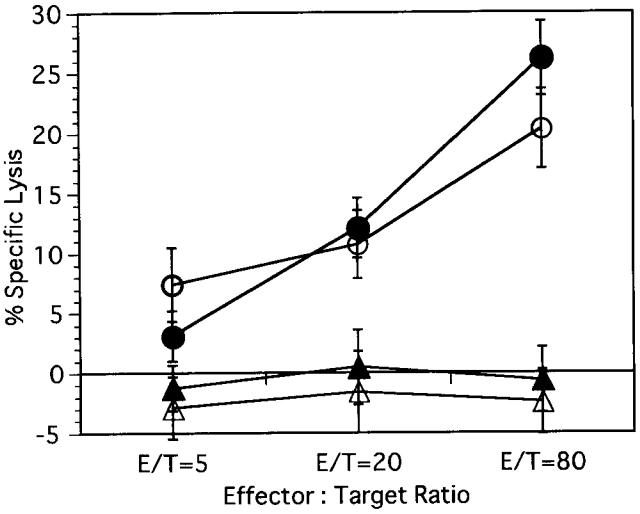

Fig. 1. HIV-1 Nef-specific CTL responses. CTL responses of target cells pulsed with synthesized peptides, GVRYPLTFGWCYKLVP (•) and QVPLRPMTYK (○), were investigated. Control DNA-injected mice (△) and nonpeptide-pulsed mice (▲) showed no CTL responses.

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